

# THE GENETIC ANALYSIS OF AN UNUSUAL RELATIONSHIP BETWEEN SELF-STERILITY AND SELF-FERTILITY IN NICOTIANA<sup>1</sup>

EDGAR ANDERSON

*Geneticist to the Missouri Botanical Garden*

*Associate Professor in the Henry Shaw School of Botany of Washington University*

AND

DOROTHEA DE WINTON

*of the John Innes Horticultural Institution*

A number of crosses between *Nicotiana alata*, *N. Sanderae*, and *N. Langsdorffii* were grown at the John Innes Horticultural Institution from 1923 to 1926 by the junior author. In most of these crosses the inheritance of self-sterility was quite straightforward, and the results obtained were substantially the same as those reported by East ('19), and East and Yarnell ('29). That is, the first generations consisted entirely of self-fertile plants, but on crossing the F<sub>2</sub> plants *inter se*, self-sterility reappeared in certain of the second-generation families. Among the plants of *N. alata* there was, however, a single plant (No. 15-2) whose cross-sterility relationships were exceptional and which gave rise to exceptional families when crossed with *N. Langsdorffii*. It is with the behaviour of No. 15-2 and its progeny that this paper is concerned. The results obtained from 1923 to 1926 with these plants are presented graphically in fig. 1, where they are contrasted with the results normally obtained upon crossing *N. alata* and *N. Langsdorffii*. Attention is called to the following peculiarities of the cross with No. 15-2: (1) The original exceptional plant was incompatible as a female with *N. Langsdorffii* though compatible as a male; (2) The F<sub>1</sub> was composed of self-sterile and self-fertile plants in approximately equal numbers—it will be remembered that normally in crosses between *N. alata* and *N. Langsdorffii* self-sterility would not appear until the second generation; (3) The self-sterile F<sub>1</sub>'s were compatible both as males and females with their self-sterile parent species *N. alata*, while with *N. Langsdorffii* they were compatible as males but incompatible as females.

<sup>1</sup> Much of the work reported in this paper was carried on under a National Research Fellowship in the Biological Sciences.



That is, they were *cross-sterile with their self-fertile parent and cross-fertile with their self-sterile parent*. No. 15-2 gave similar exceptional results when crossed with another self-fertile strain of tobacco, an ornamental garden variety of unknown ancestry obtained from Mr. E. A. Bowles. It bore small dark red flowers and was probably a self-fertile segregate from the cross *N. Langsdorffii*  $\times$  *N. Forgetiana*.

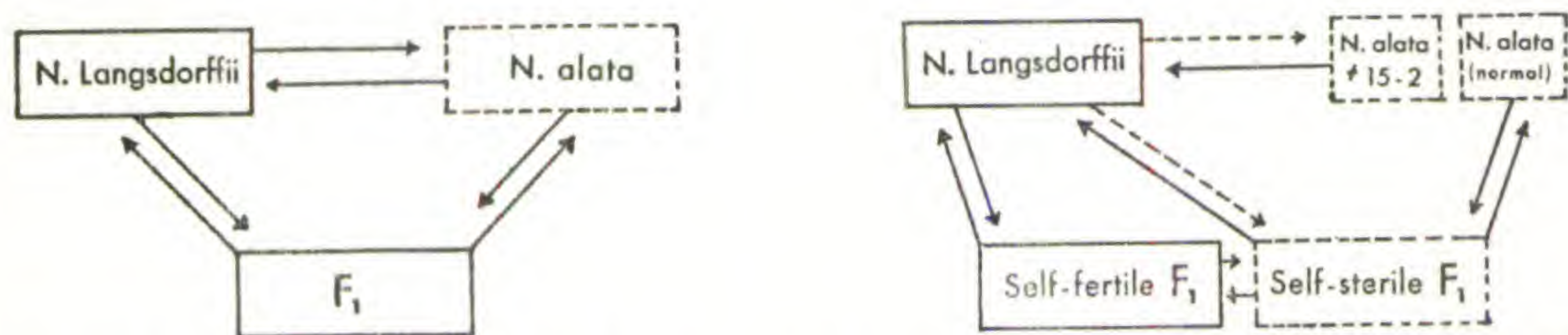


Fig. 1. Sterility and fertility relationships in normal crosses (left) and in hybrids with *N. alata* No. 15-2 (right). Solid lines indicate self- or cross-fertility; dotted lines, cross- or self-sterility.

All of these complications can be explained if we assume that No. 15-2 carried one self-sterility allelomorph of a slightly different nature from those normally present in *N. alata*. Their behavior is outlined by East and Yarnell ('29) as follows: "The action of these allelomorphs was such that a plant of constitution  $S_1S_2$ , when pollinated by pollen from a plant of constitution  $S_2S_3$ , produced two types of progeny,  $S_1S_3$  and  $S_2S_3$ , due to the slow growth of the pollen tubes bearing the factor  $S_2$ ." Continuing the notation developed by East and Mangelsdorf ('25) and East and Yarnell ('29), we may designate the exceptional allelomorph in No. 15-2 as  $S_F$ . This allelomorph operates like those designated by East and his students, but has the additional property of inhibiting the growth of pollen carrying the full fertility allelomorph  $S_f$  as well as that carrying  $S_F$ . Plant No. 15-2 was heterozygous for  $S_F$  and would set no seed with any pure self-fertile plant, since such pollen (all carrying  $S_f$ ) would not grow fast enough to cause fertilization.

The other allelomorph of 15-2 was a normal self-sterility allelomorph. In the absence of precise tests with East's material we cannot tell just which one it was but may designate it  $S_n$  to indicate any one of the self-sterility allelomorphs. No. 15-2 is therefore represented in fig. 2 as of the genetic constitution  $S_F S_n$ .







*Langsdorffii*, they would be cross-sterile, since the  $S_F$  gene would stop the  $S_f$  pollen precisely in the same way as it did in No. 15-2. On the other hand, when these self-steriles were pollinated with normal *N. alata* they would be cross-fertile. All these relationships are illustrated diagrammatically in fig. 2.

The above explanation therefore fitted all the known data and could be tested in several ways; four of these seemed worth trying. (1) A cross between a self-sterile  $F_1$  and a self-fertile  $F_1$  should give

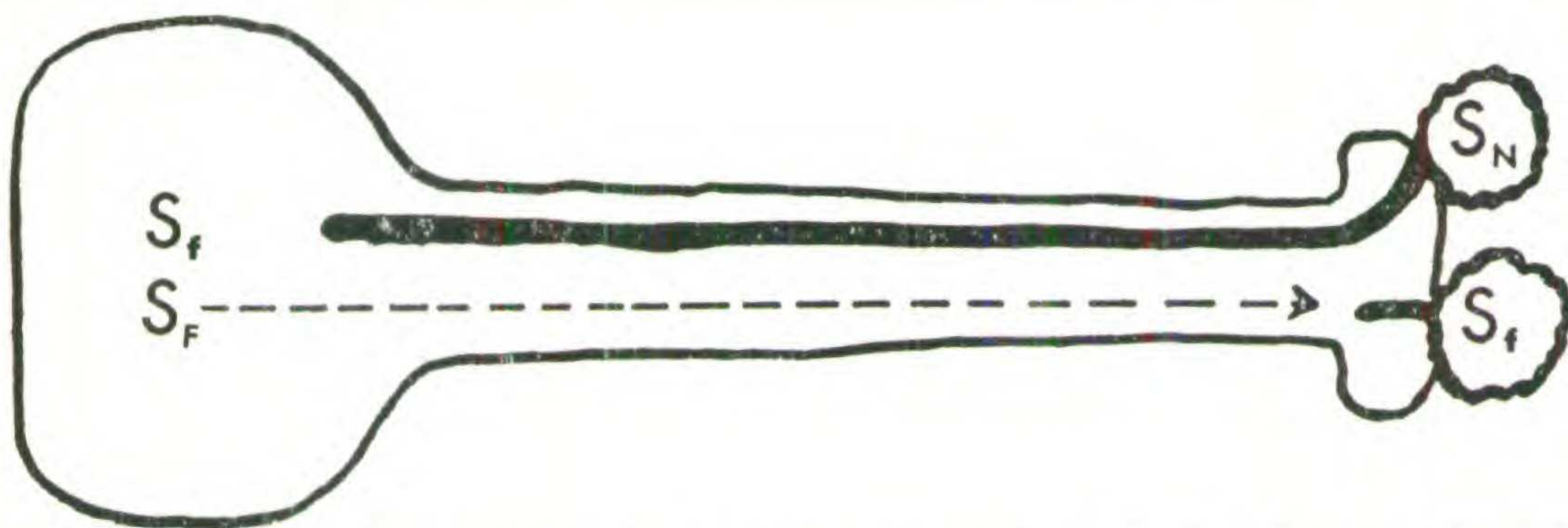


Fig. 3. Diagram of a self-sterile  $F_1$  pistil pollinated with pollen from a self-fertile  $F_1$ . Dotted line shows antagonism between factors of the style and pollen. Cross fertile.

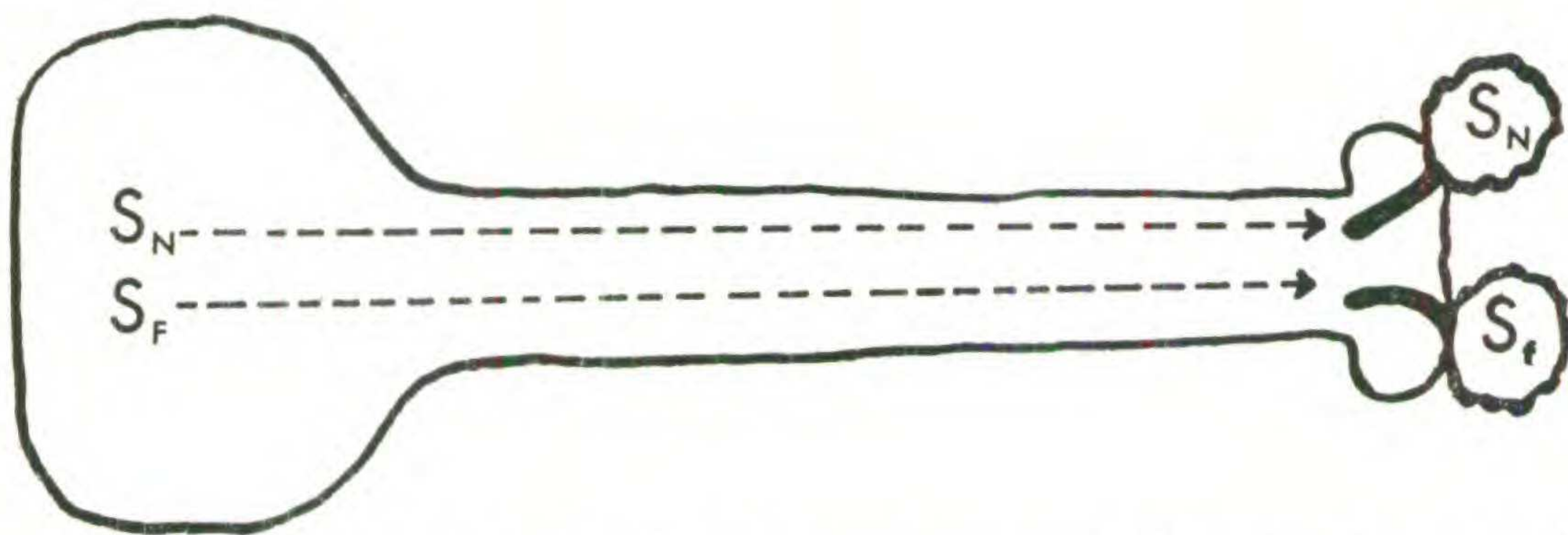


Fig. 4. Diagram of a self-sterile  $F_2$  pistil pollinated with pollen from a self-fertile  $F_2$ . Dotted line shows antagonism between factors of the style and pollen. Cross sterile.

self-fertiles and self steriles in the  $F_2$ . (2) Though the self-sterile  $F_1$ 's had been cross-fertile when pollinated by their self-fertile siblings the  $F_2$  self-steriles should be cross-sterile when pollinated by their self-fertile siblings. These relations are diagrammed in figs. 3 and 4. Family 5N-30 (the result of pollinating self-sterile  $F_1$ , No. 29-12, by its self-fertile sibling No. 29-17) was grown to test these hypotheses. They were completely verified as shown in



TABLE I  
POLLINATIONS ON SELF-FERTILE PLANTS

Plant No.	Fertile Matings			Sterile Matings		
	Self-pollinated	Pollinated by self-fertiles	Pollinated by self-steriles	Self-pollinated	Pollinated by self-fertiles	Pollinated by self-steriles
1	4					
2	4					
3	4					
4	4					
5	2					
7	4					
8	4					
9	3					
11	4					
12	3			1		
13	4					
18	4	4	2			
19	2			1		
20	2					
21	4					
22	2					
24	4					
25	3					
26	4					
27	4					
29	4					
30	4					
31	3					
32	4					
33	4					
35	4					
37	1					
38	4					1
40	2			2		
41	2					
42	4					
43	3					
44	3					

POLLINATIONS ON SELF-STERILE PLANTS

6			1	4		
10				3		
14			1	5		2
15				7	16	13
16		2	1	8	5	2
17		2	3	4	5	11
23				3		
28		5	7	7		11
34				4		
36				3		
39	1			3		1
45				4		
46				4		
49				4		



table I. There were thirty-four self-fertiles and fifteen self-steriles. The self-steriles were furthermore not only cross-sterile when pollinated *inter se*, but were also cross-sterile when pollinated by their self-fertile siblings as is set out in the table.<sup>1</sup> (3) The self-sterile plants of the second generation, since they were carrying the factor  $S_F$ , should be sterile when pollinated by *N. Langsdorffii*. This prediction was also realized. Two unrelated strains of *Langsdorffii* were grown for the purpose. One, Family 1N-30, was from the same strain which had previously been used in the experiments. The other, Family K-30, consisted of six plants obtained from Kew Gardens. Both strains gave identical results. Both were fertile when pollinated with the  $F_2$  self-steriles and both were cross-sterile when their pollen was used *on* the self-steriles. The data are summarized in table II. (4) The self-sterile  $F_2$

TABLE II  
RESULTS OF POLLINATING SELF-FERTILE AND SELF-STERILE  $F_2$ 'S WITH  
*N. LANGSDORFFII*

	Fertile matings	Sterile matings
Self-fertile		
No. 2.....	4	0
No. 38.....	4	0
Self-sterile		
No. 14.....	0	1
No. 15.....	0	3
No. 16.....	0	4
No. 17.....	0	4
No. 28.....	0	6
No. 36.....	0	1

plants on the above hypothesis should (like the original *alata* grandparent No. 15-2) be heterozygous for  $S_F$ . Like it, therefore, when their pollen was used upon *N. Langsdorffii* they should yield progenies composed of self-sterile and self-fertile plants in approximately equal numbers ( $S_F S_f \times S_F S_n \rightarrow S_F S_f + S_f S_n$ ). The appropriate pollinations were made at the John Innes Horticultural Institution, and several families were grown at the Missouri

<sup>1</sup> It must be remembered, of course, that Family 5N-30 was an  $F_2$  from a very "wide" cross, and that, due to the recombination of modifying factors, cross- and self-incompatibility relationships could not be as clear cut as they would be in  $F_1$ , or in back-cross families. This whole matter is discussed below under the heading of "Modifying Factors."



Botanical Garden and at Washington University during the winter of 1930–1931. The results conformed completely with expectations and are briefly summarized in table III. With these results

TABLE III  
RESULTS OF CROSSING N. LANGSDORFFII AND F<sub>2</sub> SELF-STERILES

Number of self-fertile plants.....	18
Number of self-sterile plants.....	8

the interpretation given on page 98 is thought to be well-established, and no further work is planned with this material. One additional test which *might* have been tried may be pointed out, since it indicates the complexities introduced by the factor S<sub>F</sub>. A cross between a self-fertile F<sub>2</sub> and a self-sterile F<sub>2</sub> should give all self-steriles, in two intra-sterile, inter-fertile classes (S<sub>f</sub>S<sub>n</sub> × S<sub>F</sub>S<sub>n</sub> = S<sub>f</sub>S<sub>F</sub> and S<sub>n</sub>S<sub>F</sub>). A cross between these classes should produce both self-fertiles and self-steriles. In other words, on inbreeding, S<sub>F</sub> produces a bewildering maze of interweaving classes in which self-sterility seems to be dominant to self-fertility, and self-fertility dominant to self-sterility, as the following examples show:

$$\begin{array}{lcl} \begin{array}{l} \text{S. fert.} \\ \text{S}_f\text{S}_f \end{array} \times \begin{array}{l} \text{S. ster.} \\ \text{S}_F\text{S}_n \end{array} & \longrightarrow & \begin{array}{l} \text{S. fert.} \\ \text{S}_f\text{S}_n \end{array} \text{ \& } \begin{array}{l} \text{S. ster.} \\ \text{S}_f\text{S}_F \end{array} \\ \begin{array}{l} \text{S. fert.} \\ \text{S}_f\text{S}_n \end{array} \times \begin{array}{l} \text{S. ster.} \\ \text{S}_F\text{S}_n \end{array} & \longrightarrow & \begin{array}{l} \text{S. ster.} \\ \text{S}_f\text{S}_F \end{array} \text{ \& } \begin{array}{l} \text{S. ster.} \\ \text{S}_n\text{S}_F \end{array} \\ \begin{array}{l} \text{S. ster.} \\ \text{S}_f\text{S}_F \end{array} \times \begin{array}{l} \text{S. ster.} \\ \text{S}_n\text{S}_F \end{array} & \longrightarrow & \begin{array}{l} \text{S. fert.} \\ \text{S}_f\text{S}_n \end{array} \text{ \& } \begin{array}{l} \text{S. ster.} \\ \text{S}_F\text{S}_n \end{array} \end{array}$$

MODIFYING FACTORS

In so far as numbers and types of classes are concerned, the observed results are in strict accord with theoretical expectations. However, when we consider the ratios in which these different types appear, there is a wider departure from expectations. Nor is this at all surprising. Whether a plant shall be self-fertile or self-sterile is determined by the rate of pollen-tube growth in its style, and this is an exceedingly delicate reaction. Environmental



conditions affect it; East ('19) and Brieger ('27) have isolated genetic modifiers which change self-steriles into self-fertiles. If anything is at all surprising about the matter, it is the fact that, in a cross between parents differing in so many factors, and in dealing with a character so delicately adjusted as self-sterility, we should be able to find any genetic factors so clear-cut in their effect that their inheritance can be traced and their behavior in future generations confidently predicted.

As far as genetic modifiers are concerned, we should expect to get the greatest complexities in the second generation, for there recessive modifiers from either parent species would have a chance to recombine and turn otherwise self-sterile plants into self-fertiles, or vice versa. Next most difficult would be the back-crosses, while in the first generation we would expect the fewest complications. The results as reported below accord with these expectations. The only serious deviations from expectations are in the case of the second generation and in the back-cross to *N. Langsdorffii*.

In addition to the families grown in 1930 and 1931, there are the older records from 1924-26. These will all be considered together. In all the work pollinations were made in quadruplicate and when conflicting results were obtained the pollinations were repeated. The data will be discussed in the following order:

First generation—*N. Langsdorffii*  $\times$  *N. alata*

Back-crosses to *N. alata*

*N. alata*  $\times$  self-fertile  $F_1$ 's

*N. alata*  $\times$  self-sterile  $F_1$ 's

Second generation

Self-fertile  $F_1$   $\times$  self-fertile  $F_1$

Self-fertile  $F_1$   $\times$  self-sterile  $F_1$

Self-sterile  $F_1$   $\times$  self-fertile  $F_1$

Back-crosses to *N. Langsdorffii*

FIRST GENERATION—*N. LANGSDORFFII*  $\times$  *N. ALATA*

Data are at hand only for the original exceptional  $F_1$ , the result of pollinating *N. Langsdorffii* with the pollen of No. 15-2. We should expect self-fertiles ( $S_f S_n$ ) and self-steriles ( $S_F S_f$ ) in equal



numbers. The actual figures were twelve self-fertiles, sixteen self-steriles. The similar cross *Nicotiana* var. "Bowles"  $\times$  *N. alata* 15-2 also yielded self-fertiles and self-steriles in approximately equal numbers.

BACK-CROSSES TO *N. ALATA*

*N. alata*  $\times$  self-fertile  $F_1$ 's.—Two families were grown in successive years, the results of pollinating two *N. alata* siblings by two different self-fertile  $F_1$ 's. We should expect self-fertiles and self-steriles in equal numbers from such a mating. The actual results were as follows:

	$(S_xS_y) \times (S_fS_n)$	Number of self-fertile plants $S_fS_x$ & $S_fS_y$	Number of self-sterile plants $S_nS_y$ & $S_nS_y$
Family 8—1925	<i>N. alata</i> No. 35-1 $\times$ 29-7	26	17
Family 8—1926	<i>N. alata</i> No. 35-7 $\times$ 29-22	25	24

Cross- and self-fertility relationships could not be classified completely for Family 9-26, a cross between self-fertile  $F_1$  No. 29-22 and *N. alata* No. 35-4, because it was segregating for an extreme form of male sterility in which no pollen was formed by some of the plants.

*N. alata*  $\times$  self-sterile  $F_1$ 's.—Four such families were grown. In each case we should expect self-fertiles and self-steriles in equal numbers. The results are as follows:

		Number of self-fertile plants	Number of self-sterile plants
Family 16—1925	29-6 $\times$ <i>N. alata</i> No. 35-2	19	22
Family 16—1926	29-8 $\times$ <i>N. alata</i> No. 35-2	24	20
Family 17—1925	<i>N. alata</i> No. 35-1 $\times$ 29-6	20	20
Family 17—1926	<i>N. alata</i> No. 35-1 $\times$ 29-8	19	17

SECOND GENERATION

Self-fertile  $F_1 \times$  self-fertile  $F_1$ .—According to our interpretation, the genetic formula for the self-fertiles of the first generation was  $S_fS_n$ . On self-fertilization, or crossed with another self-fertile  $F_1$ , they should therefore have given all self-fertiles.

$$S_fS_n \times S_fS_n \longrightarrow S_fS_f + S_fS_n$$



This is on the hypothesis that the presence of the factor  $S_n$  in the tissues of the style will retard the growth of  $S_n$  pollen quite as effectively in a self-fertile plant as it would in a self-sterile plant. In case it did not do so a certain percentage of  $S_n S_n$  zygotes would result. As a matter of fact, two self-steriles did appear among some 200 seedlings, but in the absence of any precise data we can do no more than suggest that they may have arisen in this manner.

*Self-fertile  $F_1 \times$  self-sterile  $F_1$  and Self-sterile  $F_1 \times$  self-fertile  $F_1$ .*—In each of these cases we should expect (in the absence of modifying factors) self-steriles and self-fertiles in equal numbers. In the first we should expect the following:

$$S_r S_n \times S_F S_f \longrightarrow \begin{array}{cc} S_r S_F & S_r S_r \\ \& & + & \& \\ S_F S_n & S_r S_n \end{array}$$

In the reciprocal cross,  $S_F$  would inhibit the pollen tubes carrying  $S_r$  with the following result:

$$S_F S_f \times S_r S_n \longrightarrow S_F S_n + S_r S_n$$

Five families were grown. The first two are from a self-fertile  $F_1 \times$  a self-sterile  $F_1$ , the last three from the reciprocal combination.

		Number of self-fertile plants	Number of self-sterile plants
14—1925	29-7 $\times$ 29-12	29	9
14—1926	29-7 $\times$ 29-12	18	2
15—1925	29-12 $\times$ 29-7	27	13
15—1926	29-8 $\times$ 29-7	17	3
5N—1930	29-12 $\times$ 29-17	33	14
		—	—
		124	41
Expectations on the hypothesis outlined below		124	41

In each case there is a serious deficiency of self-steriles. Since the general situation seems to be the same in all five families, we may treat with the total of 124 self-fertiles and 41 self-steriles. If there had been no complications we should have obtained 82 of each. Two recessive modifiers, however, such as those already described by East ('19), would have so changed part of the 82 self-steriles that in the absence of precise pollination tests with



plants of known constitution they would have been classified among the self-fertiles. On this interpretation, if we let *r* and *r*<sub>1</sub> represent the two recessive modifiers, *N. Langsdorffii* would have been of the constitution *S<sub>f</sub>S<sub>f</sub>rrr<sub>1</sub>r<sub>1</sub>* and *N. alata* 15-2 of the constitution *S<sub>F</sub>S<sub>n</sub>RRR<sub>1</sub>R<sub>1</sub>*. The first generation self-fertiles would therefore have been of the constitution *S<sub>f</sub>S<sub>n</sub>RrR<sub>1</sub>r<sub>1</sub>*, and their self-sterile siblings of the constitution *S<sub>F</sub>S<sub>n</sub>RrR<sub>1</sub>r<sub>1</sub>*. The cross between self-steriles and self-fertiles would be diagrammed as follows:

Self-sterile F <sub>1</sub> × Self-fertile F <sub>1</sub>	Self-fertile combinations	Self-sterile combinations
<i>S<sub>F</sub>S<sub>f</sub> Rr R<sub>1</sub>r<sub>1</sub> × S<sub>f</sub>S<sub>n</sub> Rr R<sub>1</sub>r<sub>1</sub></i>	<i>S<sub>f</sub>S<sub>n</sub> RR R<sub>1</sub>R<sub>1</sub> — 1</i> <i>S<sub>f</sub>S<sub>n</sub> Rr R<sub>1</sub>R<sub>1</sub> — 2</i> <i>S<sub>f</sub>S<sub>n</sub> RRR<sub>1</sub>r<sub>1</sub> — 2</i> <i>S<sub>f</sub>S<sub>n</sub> RrR<sub>1</sub>r<sub>1</sub> — 4</i> <i>S<sub>f</sub>S<sub>n</sub>rrR<sub>1</sub>R<sub>1</sub> — 1</i> <i>S<sub>f</sub>S<sub>n</sub>rrR<sub>1</sub>r<sub>1</sub> — 2</i> <i>S<sub>f</sub>S<sub>n</sub>RRr<sub>1</sub>r<sub>1</sub> — 1</i> <i>S<sub>f</sub>S<sub>n</sub>Rrr<sub>1</sub>r<sub>1</sub> — 2</i> <i>S<sub>f</sub>S<sub>n</sub>rrr<sub>1</sub>r<sub>1</sub> — 1</i>	<i>S<sub>F</sub>S<sub>f</sub> RR R<sub>1</sub>R<sub>1</sub> — 1</i> <i>S<sub>F</sub>S<sub>f</sub> Rr R<sub>1</sub>R<sub>1</sub> — 2</i> <i>S<sub>F</sub>S<sub>f</sub> RR R<sub>1</sub>r<sub>1</sub> — 2</i> <i>S<sub>F</sub>S<sub>f</sub> Rr R<sub>1</sub>r<sub>1</sub> — 4</i>  Total self-steriles 9
Modified self-steriles	<i>S<sub>F</sub>S<sub>f</sub>rrR<sub>1</sub>R<sub>1</sub> — 1</i> <i>S<sub>F</sub>S<sub>f</sub>rrR<sub>1</sub>r<sub>1</sub> — 2</i> <i>S<sub>F</sub>S<sub>f</sub>RRr<sub>1</sub>r<sub>1</sub> — 1</i> <i>S<sub>F</sub>S<sub>f</sub>Rrr<sub>1</sub>r<sub>1</sub> — 2</i> <i>S<sub>F</sub>S<sub>f</sub>rrr<sub>1</sub>r<sub>1</sub> — 1</i>	
Total self-fertiles and pseudo self-fertiles.....		23

We should therefore expect a ratio of 23 self-fertiles to 9 self-steriles. That is, out of every 32 plants we would expect one-half to be true self-fertiles. The other half would be divided into 9 self-steriles and 7 modified self-steriles which would be fertile with their own pollen, but cross-sterile in certain combinations. For 165 plants the expectations of a 23:9 ratio are (in whole numbers) 124 to 41, which is the exact number actually obtained.

BACK-CROSSES TO *N. LANGSDORFFII*

This hypothesis can be tested by examining the back-crosses. Clearly, such modifiers could not have come from *N. alata*, since the ratios obtained in back-crosses to that species were quite as regular as in the first generation. Therefore to test our hypothesis we turn to back-crosses between an F<sub>1</sub> self-sterile and *N. Langs-*



*dorffii*. In the absence of modifying factors we should again obtain a 1:1 ratio between self-fertiles and self-steriles.

$$S_F S_f \times S_F S_f \longrightarrow S_F S_f + S_f S_f$$

If two recessive modifiers are present, we would obtain a ratio of seven self-fertiles to one self-sterile. The data are available from one such family and can be summarized as follows:

	Number of self-fertile plants	Number of self-sterile plants
Family 18—1926 26-3 $\times$ <i>N. Langsdorffii</i> No. 29-8 Expectations on hypothesis outlined above	30 (29)	3 (4)

While the data are too meagre for final conclusions to be drawn the results from the one back-cross family are consistent with the results from the second generation families. Both point to *N. Langsdorffii* as having introduced recessive modifiers into the cross, which upon recombination in the second generation and in the back-cross to *N. Langsdorffii*, turned nominal self-sterile individuals into apparent self-fertiles. The ratios from both types of families are consistent with the hypothesis that two such recessive modifiers were introduced from *N. Langsdorffii*.

#### LINKAGE BETWEEN THE SELF-STERILITY ALLELOMORPHS AND OTHER GENES

Family No. 5N-30 was a second generation from a cross between *N. Langsdorffii* and *N. alata*. These two species (or sub-species) differ by a large number of other characters beside self-sterility and self-fertility. Figure 5 and pl. 4 show typical flowers of each species. Some of the most outstanding differences are set out below in tabular form:

<i>N. Langsdorffii</i>	<i>N. alata</i>
Flowers green	Flowers white
Corolla-tube short	Corolla-tube long
Style proportionately short	Style proportionately long
Pollen blue	Pollen ivory



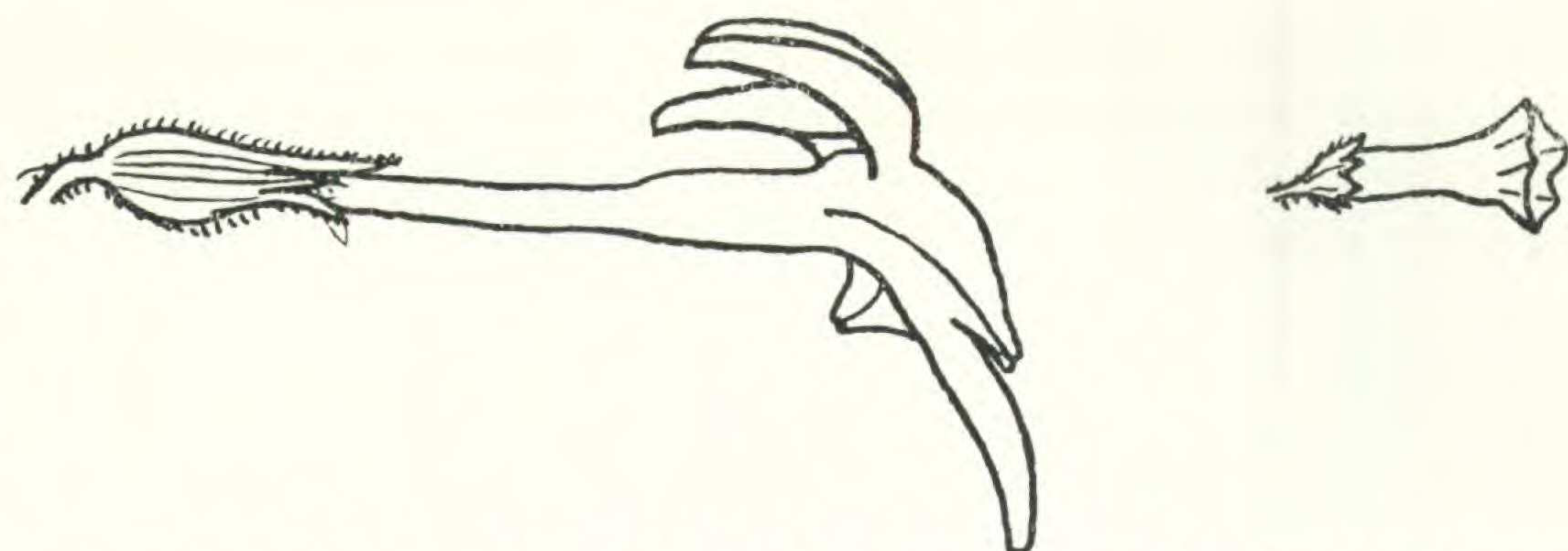


Fig. 5. Typical flower of *N. alata* (left) and of *N. Langsdorffii* (right) drawn to the same scale.

Nor are there any evident cytological complications which would hinder free recombination. Examination of the pollen mother cells with iron-aceto-carmin showed the reduction divisions to be regular. Certainly there was not more irregularity than existed in either of the parent species. Since the parent species differed in such a large number of characters it was thought probable that some of them would show linkage with the self-sterility allelomorphs. Such linkage has been reported for *Nicotiana* by Brieger and Mangelsdorf ('27) (anthocyanin flower color) and for *Antirrhinum* by Brieger ('30). Each plant of Family 5N-30 was accordingly scored for flower color, pollen color, tube length, and style length.

*Corolla color.*—As has been determined by a number of investigators the difference between the green-plastid corolla of *N. Langsdorffii* and the pure white-plastid corolla of *N. alata* is mainly due to a single factor, green being a simple dominant to white. The data

TABLE IV  
NUMBER OF PLANTS OBTAINED IN A SECOND-GENERATION CROSS OF GREEN SELF-FERTILE AND WHITE SELF-STERILE

	Number of self-fertile plants	Number of self-sterile plants	Total
Green plastids	26 (25.3)*	10 (10.7)	36
White plastids	7 (7.7)	4 (3.3)	11
Total	33	14	47

\* The figures outside the parentheses indicate the actual number of plants obtained. The figures within the parentheses show the number to be expected if self-sterility is inherited independently from plastid color.



from 5N-30 were in accord with this interpretation and also indicated that there is no appreciable linkage between the factor for plastid color and the self-sterility allelomorphs,  $S_1$ ,  $S_2$ ,  $S_f$ , etc. The data are given in table IV.

*Corolla-tube length.*—As is shown in fig. 5 and in pl. 4, this is the most conspicuous difference between the two parental species. It is due to at least four or five main pairs of factors and a number of minor modifying factors. Since there are only nine pairs of chromosomes in *N. alata* and *N. Langsdorffii*, the chances are good that at least one pair of factors affecting tube length might be linked with the self-sterility allelomorphs. From purely *a priori* assumptions we should therefore expect, upon crossing the long-tubed self-sterile species with the short-tubed self-fertile one, to find a higher percentage of self-steriles among the longest-tubed members of the second generation than among the shorter-tubed ones. The actual figures are as follows:

Tube length in millimeters	Number of self-fertile plants	Number of self-sterile plants	Per cent of self-sterile plants
30-39	12	3	20
40-49	16	6	27
50-59	5	5	50

*Style length.*—In *N. Langsdorffii* the style is shorter than the tube; in *N. alata* it is often much longer and the protruding stigmas are very conspicuous. While this is a highly variable character, even on a single plant, it can be roughly classified for purposes of comparison. The plants of 5N-30 were recorded as short-styled (like *N. Langsdorffii*) or long-styled (like *N. alata*) or intermediate. From the behavior of the character in later generations it is clearly affected by several pairs of factors. It is not surprising therefore that a higher percentage of the longer-styled plants were self-sterile, as the following table shows:

Proportional length of style	Number of self-fertile plants	Number of self-sterile plants	Per cent of self-sterile plants
Short	11	2	15
Intermediate	4	1	20
Long	18	11	38



*Pollen color.*—The pollen of *N. Langsdorffii* is a bright dark blue, that of *N. alata* is ivory or cream-colored, though the stamens themselves are often dark. The pollen of the F<sub>1</sub> plants was intermediate in color. In the second generation dark blue, ivory, and intermediates resembling the F<sub>1</sub> could be distinguished. The segregation is fairly clear-cut; probably not more than two or three pairs of factors are involved. One of them is evidently quite strongly linked with the sterility allelomorphs. It will be seen that we did not obtain a single self-sterile plant with dark blue pollen.

Pollen color	Number of self-fertile plants	Number of self-sterile plants	Per cent of self-sterile plants
Blue	7	0	0
Intermediate	19	7	27
Ivory	7	7	50

From the above discussion it is clear that in *Nicotiana* a number of genes are linked closely enough with the self-sterility allelomorphs to be detected readily. Linkage has been demonstrated and the linkage intensity calculated for the gene for anthocyanin flower color by Brieger and Mangelsdorf ('26). In the data reported above the linkage of one of the genes controlling pollen color and of at least one each of the genes for tube length and for proportional length of style is indicated. A careful study of other multiple factor differences between *N. Langsdorffii* and *N. alata* (as, for instance, leaf shape and stipule decurrence) would greatly extend the list.

For all of these genes Family 5N-30, a cross between two first-generation plants, was more like an ordinary back-cross than a normal second generation, due to the influence of the self-sterility allelomorphs. This fact is brought out diagrammatically in fig. 6, where the relationships of genes and the proportional contributions of the two parental species to the second generation are diagrammed, first, for an ordinary pair of Mendelian factors, and second, for the self-sterility allelomorphs S<sub>F</sub>, S<sub>f</sub>, etc. In the case of ordinary genes, if we consider the second generation as a whole, the two parental species have made equal contributions. In the case of the self-sterility allelomorphs all the male gametes carrying the S<sub>f</sub> factors from *N. Langsdorffii* have been stopped.



As a result three-quarters of the self-sterility genes of the second generation, considered as a whole, have had their origin from *N. alata* and only one quarter from *N. Langsdorffii*. As far as the self-sterility allelomorphs are concerned the second generation Family 5N-30 was a back-cross to *N. alata*. To a lesser extent this was true as well for all the genes linked with the self-sterility allelomorphs.

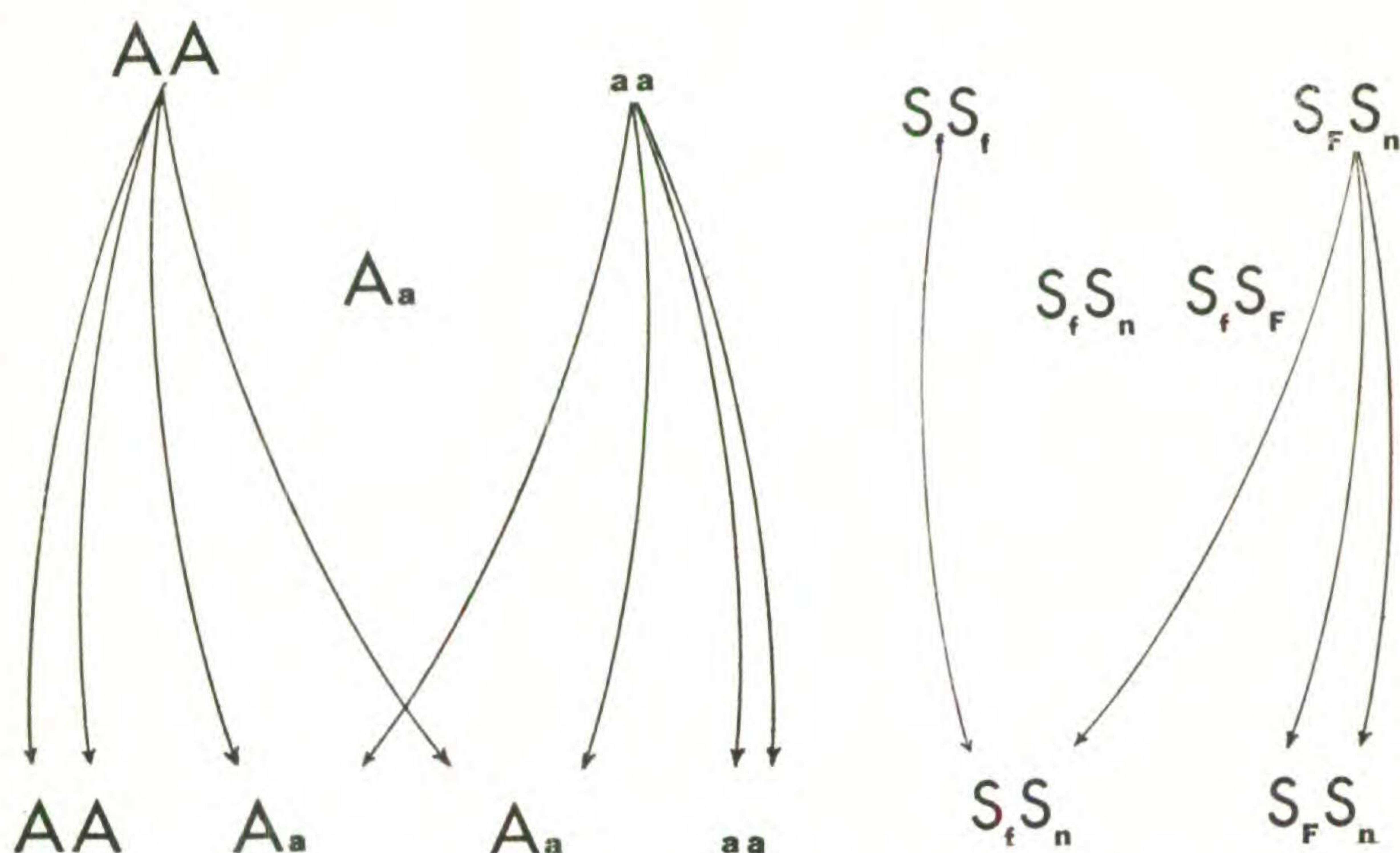


Fig. 6. Proportional contributions to the F<sub>2</sub>: in the case of an ordinary pair of Mendelian factors (left); and in the case of the self-sterility allelomorphs (right).

The net result of such linkage should be quite striking in a species cross. The magnitude of the effect would depend upon the ratio between the non-crossed-over chromosome segment carrying the S factor and the chromatin as a whole. If we let  $n$  represent the average number of such segments then  $n-1$  will represent that part of the chromatin not linked with the S factors. In the second generation this much of the chromatin will segregate normally and the relative contributions of the two species will be equal. If we let  $L$  stand for *Langsdorffii* chromatin and  $A$  for *alata* chromatin, the composition of the second generation can be represented as  $2(n-1)L + 2(n-1)A$ . For the segment containing the S factors, segregation will be abnormal. If the cross was made



as in the case of Family 5N-30, the composition of the second generation for this segment of chromatin will be  $1L + 3A$ . Combining the two totals, the proportion of L and A chromatin in the second generation will be  $2(n-1)L + 1L : 2(n-1)A + 3A$  which simplifies to  $(2n-1)L : (2n+1)A$ . In the absence of any genetical or cytological data as to the frequency of crossing-over in *Nicotiana* we can go no further with certainty. We know, however, that there are nine pairs of chromosomes. If, just as a guess, we take the average cross-over length as one-third of a chromosome,  $n$  will equal twenty-seven and the proportion of L and A chromatin in Family 5N-30 will be  $53L : 55A$ .

That this is no mere idle speculation is proved by a comparison of East's ('16) tables and plates with those presented in this paper. In each case a second generation between *N. alata* and *N. Langsdorffii* was studied. In his case the selective effect of the S factors, if any, was in favor of *N. Langsdorffii*. In the cross reported here it was in favor of *N. alata*. And the tables and plates show that 5N-30 was definitely more like *N. alata* than the second generation families figured by him. Precise comparison is possible only for the length of corolla-tube. The data from both crosses are assembled graphically in fig. 7. Family 5N-30 is definitely more like *N. alata* than was East's  $F_2$ . The difference in the shape of the two curves is quite as striking as their position and is equally significant.

It may be remarked in closing that the complications introduced by self-sterility factors will be more striking in the case of species crosses than in crosses between closely related strains. In the latter case such anomalies as are due to linkage with the self-sterility allelomorphs will be apparent mainly in the ratios obtained between different types of offspring. In the case of species crosses, however, the chromatin in the neighborhood of the self-sterility allelomorphs will have accumulated a whole set of differing genes in the two species. In hybrids between them, reciprocal crosses may be characterized by gross morphological differences. This possibility has been alluded to by Brieger in his recent monograph ('30) and is, according to his brief reference, the explanation of the differences obtained in reciprocal crosses in *Antirrhinum* by Lotsy ('12) and Baur ('11).



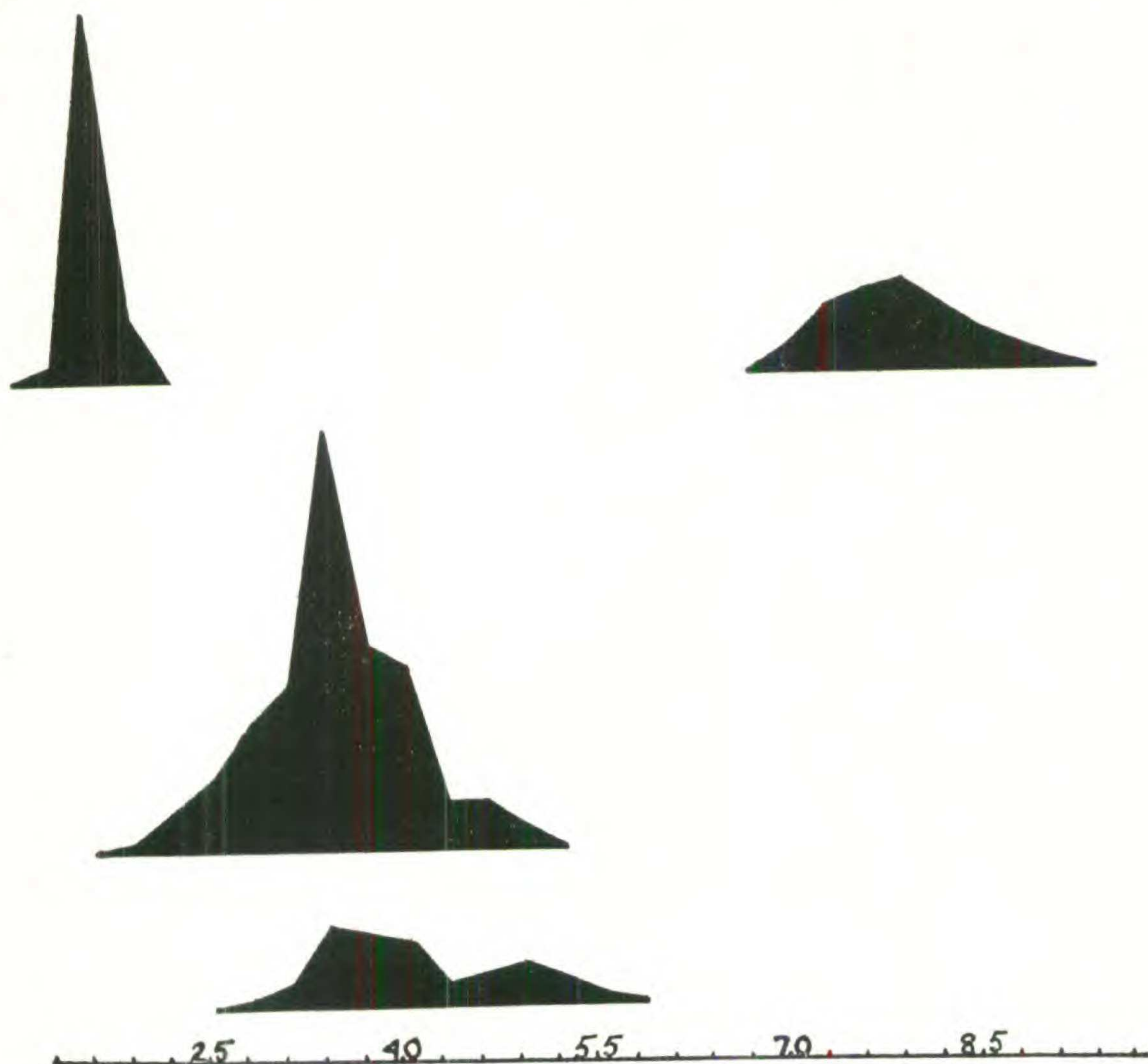


Fig. 7. Corolla length in centimeters for:

<i>N. Langsdorffii</i>	(upper left)	56 individuals	(data from East).
<i>N. alata</i>	(upper right)	49 individuals	(data from East).
East's $F_2$	(center)	163 individuals	(data from East).
Family 5N-30	(lower center)	47 individuals.	

(Each division of the scale represents 3 mm.)

#### SUMMARY

I. In a number of crosses between self-fertile and self-sterile species of *Nicotiana*, a single plant of *Nicotiana alata* gave the following anomalous results:

1. It was female sterile when pollinated with self-fertile *Nicotianas*.
2. When used as a pollen parent with self-fertile *Nicotianas* half of the first generation hybrids were self-sterile.



3. These exceptional  $F_1$  self-steriles were (as females) cross-sterile with their self-fertile parents!

II. These anomalies are interpreted as due to a single factor,  $S_F$ , belonging to the allelomorphic series  $S_f$ ,  $S_1$ ,  $S_2$ ,  $S_3$ ,  $S_4$ , etc., studied by East and his students.  $S_F$  has the same properties as the self-sterility allelomorphs previously described; that is, its presence in the cells of the style inhibits the growth of pollen tubes carrying the same factor. It differs from the factors hitherto described in that it also inhibits all pollen carrying the full fertility allelomorph  $S_f$ .

III. The  $F_1$ ,  $F_2$ , and back-cross ratios of self-fertile to self-sterile are consistent with the assumption that two recessive modifying factors were introduced into the cross from *N. Langsdorffii*. When homozygous they turn otherwise self-sterile plants into apparently self-fertile plants (pseudo-self-fertiles).

IV. The  $S$  allelomorphs were found to be independent of the factor for green corolla color (plastid color). They are linked with one of the factors for pollen color and with at least one of the factors for length of corolla tube and for proportional length of style.

Attention is called to certain complications introduced into inter-species crosses by the interaction of the self-sterility allelomorphs. The morphological differences between the *Langsdorffii-alata*  $F_2$  studied by East and the second generation family of the present experiment are interpreted on this basis.

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## EXPLANATION OF PLATE

## PLATE 4

One flower each from the plants of Family 5N-30, a second generation from the cross *N. Langsdorffii*  $\times$  *N. alata*. At the extreme left single flowers of the two parent species for comparison.